

WE CLAIM:

- Sub D1*
1. A non-reducing saccharide-forming enzyme, which forms a non-reducing saccharide having a trehalose structure as an end unit from a reducing partial starch hydrolysate, and which has an optimum temperature in a medium temperature range.
  2. The enzyme of claim 1, which has an optimum temperature of over 40°C but below 60°C.
  - Sub D2* 3. The enzyme of claim 1, which has an optimum pH in an acid pH range.
  4. The enzyme of claim 1, which comprises a part or the whole of the amino acid sequence of SEQ ID NO:1.
  5. The enzyme of claim 1, which comprises a part or the whole of the amino acid sequence of SEQ ID NO:2 or 3.
  6. The enzyme of claim 1, which comprises a part or the whole of the amino acid sequences of SEQ ID NOs:4 to 6.
  7. The enzyme of claim 1, which is derived from a microorganism.
  8. The enzyme of claim 7, wherein said microorganism is one of the genus *Arthrobacter*.
  9. The enzyme of claim 7, wherein said microorganism is a member selected from the group consisting of *Arthrobacter* sp. S34, FERM BP-6450, and mutants thereof
  10. A non-reducing saccharide-forming enzyme obtainable from a microorganism selected from the group consisting of *Arthrobacter* sp. S34, FERM BP-6450, and mutants thereof capable of producing the enzyme of claim 1, said enzyme forming a non-reducing saccharide having a trehalose structure

as an end unit from a reducing partial starch hydrolysate.

11. A non-reducing saccharide-forming enzyme obtainable by the expression of a DNA encoding the enzyme of claim 1, said enzyme forming a non-reducing saccharide having a trehalose structure as an end unit from a reducing partial starch hydrolysate.

12. A non-reducing saccharide-forming enzyme, which comprises an amino acid sequence having at least 57% homology to the amino acid sequence of SEQ ID NO:1, and which forms a non-reducing saccharide having a trehalose structure as an end unit from a reducing partial starch hydrolysate.

*See D3* 13. The non-reducing saccharide-forming enzyme of 1, which has the following physicochemical properties:

(1) Action

Forming a non-reducing saccharide having a trehalose structure as an end unit from a reducing partial starch hydrolysates having a degree of glucose polymerization of 3 or higher;

(2) Molecular weight

About  $75,000 \pm 10,000$  daltons on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE);

(3) Isoelectric point (pI)

About  $4.5 \pm 0.5$  on isoelectrophoresis using ampholyte;

(4) Optimum temperature

About  $50^{\circ}\text{C}$  when incubated at pH 6.0 for 60

min;

(5) Optimum pH

About 6.0 when incubated at 50°C for 60 min;

(6) Thermal stability

Stable up to a temperature of about 55°C when incubated at pH 7.0 for 60 min; and

(7) pH Stability

Stable at pHs of about 5.0 to about 10.0 when incubated at 4°C for 24 hours.

See  
D3  
cont

14. A DNA encoding the enzyme of claim 1.

15. The DNA of claim 14, which comprises a part or the whole of the nucleotide sequence of SEQ ID NO:7 or its complementary nucleotide sequence.

16. The DNA of claim 14, which comprises a part or the whole of the nucleotide sequence of SEQ ID NO:8.

17. The DNA of claim 14, wherein one or more bases are replaced with another bases based on the degeneracy of genetic code without altering the amino acid sequence encoded thereby.

18. The DNA of claim 14, which has been inserted into an autonomously-replicable vector.

19. The DNA of claim 14, which has been introduced into an appropriate host.

20. A process for producing a non-reducing saccharide-forming enzyme, which comprises the steps of:

culturing a microorganism, capable of forming the enzyme of claim 1, in a nutrient culture medium to form said

enzyme; and

collecting the formed enzyme from the resulting culture.

21. The process of claim 20, wherein said microorganism is one of the genus *Arthrobacter*.

22. The process of claim 20, wherein said enzyme is obtainable from a microorganism selected from the group consisting of *Arthrobacter* sp. S34, FERM BP-6450, and mutants thereof capable of producing the enzyme of claim 1.

23. The process of claim 20, wherein said microorganism is a transformant which has been prepared by introducing into an appropriate host a DNA which encodes the enzyme of claim 1.

24. The process of claim 20, which comprises the steps of:

treating the resulting culture with a cell-lysis enzyme; and

collecting the non-reducing saccharide-forming enzyme from the treated culture.

25. The process of claim 20, wherein the produced non-reducing saccharide-forming enzyme is collected by one or more techniques selected from the group consisting of dialysis, salting out, filtration, concentration, separatory sedimentation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, reverse-phase chromatography, affinity chromatography, gel electrophoresis, and isoelectrofocusing.

26. A trehalose-releasing enzyme, which specifically

hydrolyses a non-reducing saccharide having a trehalose structure as an end unit at a site between a part of the trehalose structure and a part of the resting, and which has an optimum temperature in a medium temperature range.

27. The enzyme of claim 26, which has an optimum temperature of over 45°C but below 60°C.

28. The enzyme of claim 26, which has an optimum pH in an acid pH range.

29. The enzyme of claim 26, which comprises a part or the whole of the amino acid sequence of SEQ ID NO:9.

30. The enzyme of claim 26, which comprises a part or the whole of the amino acid sequences of SEQ ID NOs:10 to 13.

31. The enzyme of claim 26, which comprises a part or the whole of the amino acid sequences of SEQ ID NOs:14 to 16.

32. The enzyme of claim 26, which is derived from a microorganism.

33. The enzyme of claim 32, wherein said microorganism is one of the genus *Arthrobacter*.

34. The enzyme of claim 32, wherein said microorganism is a microorganism selected from the group consisting of *Arthrobacter* sp. S34, FERM BP-6450, and mutants thereof.

35. A trehalose-releasing enzyme obtainable from a microorganism selected from the group consisting of *Arthrobacter* sp. S34, FERM BP-6450, and mutants thereof capable of producing the enzyme of claim 26, said enzyme specifically hydrolyzing a non-reducing saccharide having a trehalose structure as an end unit at a site between a part of the trehalose structure and a

part of the resting.

36. A trehalose-releasing enzyme obtainable by the expression of a DNA encoding the enzyme of claim 26, said enzyme specifically hydrolysing a non-reducing saccharide having a trehalose structure as an end unit at a site between a part of the trehalose structure and a part of the resting.

37. A trehalose-releasing enzyme which comprises an amino acid sequence having at least 60% homology to the amino acid sequence of SEQ ID NO:9, and specifically hydrolyses a non-reducing saccharide having a trehalose structure as an end unit at a site between a part of the trehalose structure and a part of the resting.

38. The trehalose-releasing enzyme of claim 26, which has the following physicochemical properties:

(1) Action

Specifically hydrolysing a non-reducing saccharide having a trehalose structure as an end unit at a site between a part of the trehalose structure and a part of the resting;

(2) Molecular weight

About  $62,000 \pm 5,000$  daltons on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE);

(3) Isoelectric point (pI)

About  $4.7 \pm 0.5$  on isoelectrophoresis using ampholyte;

(4) Optimum temperature

- 127 -  
153  
147

About 50°C to about 55°C when incubated at pH 6.0 for 30 min;

(5) Optimum pH

About 6.0 when incubated at 50°C for 30 min;

(6) Thermal stability

Stable up to a temperature of about 50°C when incubated at pH 7.0 for 60 min; and

(7) pH Stability

Stable at pHs of about 4.5 to about 10.0 when incubated at 4°C for 24 hours.

39. A DNA encoding the enzyme of claim 26.

40. The DNA of claim 39, which comprises a part or the whole of the nucleotide sequence of SEQ ID NO:17 or its complementary nucleotide sequence.

41. The DNA of claim 40, which comprises a part or the whole of the nucleotide sequence of SEQ ID NO:8.

42. The DNA of claim 39, wherein one or more bases are replaced with another bases based on the degeneracy of genetic code without altering the amino acid sequence encoded thereby.

43. The DNA of claim 14, which has been inserted into an autonomously-replicable vector.

44. The DNA of claim 39, which has been introduced into an appropriate host.

45. A process for producing a trehalose-releasing enzyme, which comprises the steps of:

culturing a microorganism, capable of forming the

enzyme of claim 26, in a nutrient culture medium to produce said enzyme; and

collecting the produced enzyme from the resulting culture.

46. The process of claim 45, wherein said microorganism is one of the genus *Arthrobacter*.

47. The process of claim 45, wherein said enzyme obtainable from a microorganism selected from the group consisting of *Arthrobacter* sp. S34, FERM BP-6450, and mutants thereof capable of producing the enzyme of claim 26.

48. The process of claim 45, wherein said microorganism is a transformant which has been obtained by introducing into an appropriate host a DNA which encodes the enzyme of claim 26.

49. The process of claim 45, which comprises the steps of treating the resulting culture with a cell-lysis enzyme, and collecting the trehalose-releasing enzyme from the treated culture.

50. The process of claim 45, wherein the produced trehalose-releasing enzyme is collected by one or more techniques of dialysis, salting out, filtration, concentration, separatory sedimentation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, reverse-phase chromatography, affinity chromatography, gel electrophoresis, and isoelectrofocusing.

51. A microorganism selected from the group consisting of *Arthrobacter* sp. S34, FERM BP-6450, and mutants thereof.



52. A process for producing a saccharide, which comprises the steps of:

subjecting a reducing partial starch hydrolysate to the action of the enzyme of claim 1 and/or the enzyme of claim 26 to form a non-reducing saccharide; and

collecting the produced non-reducing saccharide or a saccharide composition comprising said non-reducing saccharide from the resulting culture.

53. The process of claim 52, wherein said reducing partial starch hydrolysate is one having a glucose polymerization degree of 3 or higher and being obtainable by subjecting starch or amylaceous substance to the action of an acid and/or a starch hydrolase.

54. The process of claim 52, wherein one or more enzymes selected from the group consisting of  $\alpha$ -amylase,  $\beta$ -amylase, glucoamylase, starch-debranching enzyme, cyclomaltodextrin glucanotransferase, and  $\alpha$ -glucosidase are further allowed to act on the reducing partial starch hydrolysate in the step of forming the non-reducing saccharide.

55. The process of claim 52, wherein said non-reducing saccharide is a member selected from the group consisting of trehalose,  $\alpha$ -glucosyltrehalose,  $\alpha$ -maltosyltrehalose,  $\alpha$ -maltotriosyltrehalose,  $\alpha$ -maltotetraosyltrehalose, and  $\alpha$ -maltopentaosyltrehalose.

56. The process of claim 55, wherein said trehalose is in the form of a hydrous- or anhydrous-crystal.

Add D4